One Laboratory’s Experience with the Transition from Culture to Molecular Methods for the Detection of Methicillin-Susceptible \textit{Staphylococcus aureus} and Methicillin-Resistant \textit{Staphylococcus aureus}

By Barbara A. DeBurger and Dr. Joel E. Mortensen

The introduction of molecular testing into clinical microbiology laboratories can be a major paradigm shift. Clinical microbiology laboratories have an opportunity to replace traditional methods with molecular methods that can have numerous advantages. However, educating and fulfilling the clinical needs of physicians while complying with administrative, operational, staffing, and financial constraints can be daunting both during and after the introduction of molecular methods. In hospital settings, rapid molecular methods can play major roles in identifying patients with healthcare-associated infections (HAI) such as \textit{Clostridium difficile} infections (CDI), colonization by vancomycin-resistant enterococci and/or detection of methicillin-susceptible \textit{Staphylococcus aureus} (MSSA) and methicillin-resistant \textit{S. aureus} (MRSA). Healthcare-associated infections cause significant morbidity and mortality in both hospital and community settings\(^1\) and have been reported to result in over 6.5 billion dollars in treatment costs and excess hospital stays\(^2\).

\textit{Staphylococcus aureus} causes a significant number of infections and deaths worldwide and is a major source of HAIs. \textit{S. aureus} is found primarily as normal flora in the nose; 20-30% of the world population is long-term carriers of \textit{S. aureus}\(^3\). Prolonged hospital stays, proximity to patients with \textit{S. aureus} carriage, endogenous \textit{S. aureus} flora, and long-term exposure to antimicrobial agents can lead to carriage of MSSA and MRSA. Screening of certain populations for MSSA and MRSA has become important for therapeutic management, infection control, and epidemiological purposes. \textit{S. aureus} accounts for the majority of surgical site infections at a rate of 25%.

Nasal colonization with either MSSA or MRSA is the most important and significant risk factor for developing surgical site infections\(^4\). Colonized patients can be decolonized with mupirocin ointment in the nares twice daily and given chlorhexidine baths daily for 5 days prior to surgery\(^5-7\). The logistics of MRSA screening and prophylaxis can become complicated with patients who need to travel to a (sometimes distant) center days ahead of surgery, especially if culture-based methods are used to detect MRSA.

Traditionally, bacterial culture has been the gold standard for isolating clinically important bacteria, such as \textit{S. aureus}. Commonly used, multipurpose, commercially available media that grows most facultative anaerobic bacteria and selective media designed to enhance the growth of specific bacteria and inhibit others has been used almost universally to detect \textit{S. aureus} at 24 to 48 hours. Prior to 2003,
most clinical microbiology laboratories cultured/ screened for MRSA by inoculating non-selective Trypticase™ Soy Agar with 5% Sheep Blood and a selective medium such as Mannitol Salt Agar or Phenylethyl Alcohol Agar with 5% Sheep Blood (PEA).

Then in early 2000’s chromogenic medium from various manufacturers became available. These were selective and differential media that contained a chromogenic substrate that forms a colored compound when degraded by bacterial enzymes contained within S. aureus. If the media also contained a high concentration of methicillin, any growth would be considered methicillin-resistant, as well. Chromogenic medium reduced the need for subculture and additional biochemical and antimicrobial susceptibility testing. Reluctance to implement molecular testing for the detection of MSSA and MRSA include (a) clinical microbiologists are comfortable with solid media, (b) the time needed for a conventional culture seemed clinically acceptable, (c) the additional cost of a molecular method was too high, and (d) isolates may be needed for further testing.

The next major change in the detection of MRSA and MSSA was the introduction of molecular methods. In 2010, three molecular tests for S. aureus and/or MRSA were commercially available: Cepheid Xpert®, BD GeneOhm™ StaphSR, and Roche Lightcycler®. The Cepheid Xpert was random access; the BD GeneOhm and Roche Lightcycler were designed for batch testing and required more hands-on time. But these platforms still had a number of limitations. The sensitivities and specificities ranged from 82-100% and 64-99%, respectively8. The only FDA-cleared clinical specimen for S. aureus and MRSA screening was nares.

As Cincinnati Children’s Hospital Medical Center (CCHMC) Diagnostic Infectious Disease Testing Laboratory moved into its current molecular testing, several factors were evaluated: patient populations, clinician needs, peer reviewed studies, and impact on laboratory operations. After respiratory and stool testing was determined, what were the next high priorities - C. difficile toxin testing and MRSA. Because the laboratory had adopted BD MAX™ for stool pathogen test-
Value of Molecular Testing

An understanding of the cost of molecular testing and conventional cultures are crucial. A detailed cost comparison of these two methods is shown in Table 1. Labor costs are a significant variable; other variables are laboratory space and fixed costs associated with incubators, refrigerators, and storage requirements for supplies. A cost savings was found in labor and sample preparation of BD MAX tests. In our facility an Infection Prevention algorithm mandates all NICU patients to be screened for MSSA and MRSA on Monday morning. Approximately 50 nares swab samples are received. Additional specimens from pre-surgical patients are submitted and tested throughout the week. With culture methods, the samples were batched for plating on Chromogenic medium at specified times so the incubation time was consistently 20-24 hours; thus, the total turn-around-time was 24-36 hours. Some cultures took longer because of the need to rule out other organisms such as Corynebacterium spp., Chryseobacterium spp., Enterococcus spp., Rhodococcus spp., and Bacillus spp. that can also produce mauve colonies. The average time to receive, perform computer processes, batch inoculate and examine, perform follow-up testing, and enter results was approximately 15 minutes per culture. In contrast, BD MAX StaphSR requires about 30 minutes to process 24 samples. At CCHM, a 24-sample run is performed at least once per eight-hour shift. Results are automatically downloaded into the laboratory information system (LIS). Incorporated rules for auto-verification allow the majority of results to be reviewed and released by the LIS software immediately after testing is completed; a technologist reviews positive or aberrant results. Not only is hands-on time for resulting reduced but also the chance for transcription error is mitigated. Although the cost per molecular test exceeds the cost per culture test, the benefits of decreased hands-on time for resulting and fewer transcription errors outweigh in the costs of the two methods. Depending on when the sample was received into the laboratory, conventional culture and BD MAX StaphSR final results are available in 24 to 36 hours and 3 to 8 hours, respectively.

<table>
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<tr>
<th>Test site burden</th>
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<th>BD MAX StaphSR</th>
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<td>Total (S)</td>
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Table 1. Cost analysis of CHROMagar™ versus BD MAX™!

* units = time in minutes.
Test site burden is the cost assigned to every assay for laboratory overhead expenses.

One Platform – many tests

A possible conundrum of contemporary molecular testing is the variety of molecular assays, tests, and panels - each with its own platform. This “too many toys” problem can arise from a desire to offer extremely sensitive and specific tests for several analytes without sufficient consideration of the requirements for each of these platforms, e.g., quality control/IQCP, quality assurance, verification, validation, space for equipment and reagents, LIS interfaces, technologists’ time, etc. Therefore, a careful plan that allows the optimum balance between the technology and the need to provide a lean, efficient laboratory is needed. In addition, a well-planned approach to molecular testing will result in a minimum number of platforms in which a maximum number of molecular tests can be performed, i.e., the more tests per platform, the better. In our laboratory, the successful verification and implementation of the BD MAX EBP, Cdiff, and StaphSR assays allowed consolidation of three different tests using three different methodologies to one platform. The list below (Figure 2) shows the versatility of the BD MAX System. Not only can several assays be performed on the same platform, but different assays can be performed at the same time. In our laboratory, BD MAX StaphSR and Cdiff are performed at the same time.
Post-Analytical Considerations

A change in testing from conventional culture to molecular testing affects more than just the laboratory. For example, Infection Prevention personnel rely on the laboratory to identify patients with MRSA for isolation purposes and modification of pre-surgical prophylaxis. In addition, hospital administrators find a direct correlation between the time to detection of MRSA by molecular methods and the time a patient is released from the hospital.

After the aforementioned change from culture to molecular methods, we found turn-around time was reduced, specimens could be tested either singly or in several batch sizes as needed, testing could be performed in 2 or even 3 shifts every day, and the need for inventory control, incubators, and refrigerators was reduced. From the clinical care perspective, we tracked MSSA and MRSA colonization from NICU and pre-surgical patients for 6 months before and 6 months after implementation of BD MAX StaphSR.

Although the study sample size is currently small, preliminary results indicate more positive patients have been identified by using the BD MAX than with culture. In NICU patients, we have found 1% more MSSA and MRSA by using molecular testing. However, in pre-surgical patients, we have found molecular testing detected slightly more 1% more MSSA and MRSA compared to culture methods (Figure 3). Although the percentage increase is relatively small, because of the large denominator, the actual number of patients is important. Our results were similar to those of other studies that were summarized in a large meta-analysis of 29 combined studies which concluded that PCR methods had the highest sensitivity of all methods compared in the analysis.

Although the increased sensitivity of PCR is a major advantage, there are times when actual isolates may be required to determine genetic relatedness for epidemiological purposes. To address this need, a study performed at our facility showed that MSSA and MRSA can be isolated from the BD MAX Sample Buffer Tube (SBT). Sample buffers positive for either S. aureus or MRSA by the BD MAX were inoculated onto Mannitol Salt Agar and incubated at 35° C for 18 to 24 hours. MSSA or MRSA was recovered from 91% of the positive sample buffers (unpublished data). A second study at our institution examined the possibility of extracting S. aureus DNA from an SBT eliminating the need to grow the organism. A known amount of S. aureus ATCC 25923 DNA was diluted with sample buffer and saline and extracted by using the bioMerieux easyMag® Nuclisens®. Repeated testing supported that sample buffer does not inhibit extraction of DNA (unpublished data). Therefore, we are performing studies to determine the utility of using sample buffers for molecular epidemiology and focused sequencing purposes.
Education of Technologists and Healthcare Personnel

Before adopting a molecular platform, education of the technologists and healthcare personnel must be carefully and thoroughly provided. In the laboratory, molecular testing requires sterile technique and decontamination procedures designed to control contamination of patient specimens and testing environments by amplicons. Technologists must be trained and understand the importance of non-contaminated pre- and post-amplification areas. Environmental testing for amplicons should be performed monthly.

Beyond the laboratory, healthcare professionals should be educated to prepare them for the logistics and importance of molecular testing and how to interpret the results. Key education steps for bringing in a new molecular platform include:

- **Education of the Laboratory Staff**: Introduce the method using lab rounds, demonstrations, and “Lunch and Learn” sessions. Education should be started early and overlap with training sessions. Everyone in the laboratory needs to understand the concepts of molecular testing so that they can answer questions.

- **Education of Clinicians**: Major users of molecular tests such as surgery, intensive care units, Infection Prevention, physicians, nurses, and nurse practitioners should be educated as early as possible. Training must include discussions on collection supplies, collection methods, test ordering and resulting.

- **Education of other users**: There are other in-house groups and outside clinics and offices that may need this test. Plan one-on-one sessions or the use of Newsletters/Updates to spread the word about this new method.

- **Education of specific technologists**: One or two technologists are the key operators in the laboratory and need to receive the most training and hands-on experience. Secondary users (such as technologists in the set-up/accessioning area where the sample is processed) should receive thorough training as well. Detailed checklists for training and competency and post training tools such as quizzes can be used to document training. Online documentation available in the laboratory information system can be useful for many of these tasks.

**Conclusion**

Cincinnati Children’s Hospital Medical Center Diagnostic Infectious Disease Testing Laboratory decided to invest in the BD MAX System as a single molecular testing platform that provides testing for multiple targets. We are closely monitoring the impact of molecular testing on patient care, as we continue to adapt and integrate these new technologies into our microbiology laboratory.

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**References**

2. https://www.cdc.gov/hai/pdfs/hai/scott_costpaper